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IN-VITRO DETERMINATION OF SUN PROTECTION FACTOR AND EVALUATION OF HERBAL OILS

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ABSTRACT

The aim of this study was to determine the Sun Protection Factor of Herbal oil that can be used in sunscreen formulation. Herbal phytoconstituents act as antioxidant which reflect or absorbed UV- radiation before reaching to the skin. Sun protection factor is determined by spectrophotometric method according to Mansur et al in the UV region 290-320 nm. From the result it was found that SPF of carrot seed oil was found to be 19, SPF of wheat germ oil was found to be 22, SPF of jojoba oil was found to be 06 and SPF of olive oil was found to be 09.Higher the SPF value higher will be the UV absorption activity of sunscreen. All this oils have been used in cosmetic for various purposes like emollient, antioxidants, anti-ageing and anti-wrinkle. In future this study will be helpful for selection of herbal oil for the development of sunscreen formulation that gives better potential sunscreen effect if they would be used in combination then might it will be given synergistic sunscreen effect.

Keywords: Sun Protection Factor, Herbal oil.

INTRODUCTION

Chronic exposure to sunrays increases the risk of skin cancer. The skin is one of the largest organs in the body in surface area and weight [1]. Sun rays adversely affect the skin, harmful effects of solar radiation on the skin are premature aging or cutaneous cancer, basal cell carcinoma, sunburns, malignant melanomas [2]. Solar light consists of different radiations range are UVA in the 320-400, UVB-290-320 and UVC- 100-290 nm range respectively. Cosmetics products are apply on the body for the purpose of nourishing, cleansing, beautifying, protection or altering appearance and enhancing the beauty. Herbal cosmetics are gaining popularity due to easy avaibility, natural no or less side effects along with cosmetic and therapeutic approach, which is increase the demand of herbal cosmetics day by day throughout the world. Phytochemicals as ingredients in cosmetic formulation as they can protect the skin against exogenous and endogenous harmful agents and can help remedy for many skin conditions. Exposure of skin to sunlight and other atmospheric condition causes production of reactive oxygen species, which can react with DNA, Protein and Fatty acids causing oxidative damage and impairment of antioxidant system. The herbal phytoconstituents or herbal extracts act on this particular area and produce antioxidant effect, relieving erythema, healing, softening, rejuvenating and sunscreen effect. "The herbal cosmetics are products which are formulated using various permissible cosmetic ingredients to form the base in which one or more herbal phytoconstituents are used to provide defined cosmetic benefits". The photoprotective phytoconstituents such as silymarin, geinstein, curcumin, resveratrol, tea polyphenols, quercetin and ascorbic acid which are used for the development of herbal cosmetic formulation.

In this study four oils, that is carrot seed oil, wheat germ oil, jojoba oil, olive oil are selected to determine SPF because Daucuscarrota contain high concentration of B-carotene which is precursor of vit. A, It also contain vit.B1, B2, B5, B6, B9 and Vit E and vit C which has good antioxidant activity which prevent wrinkle and gives anti-ageing property [3-7].

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Simmondsia chinensis has been widely used for its anti-innflamatory, hair conditioner, sunscreen effect, antibacterial, antifungal, antioxidant, anti-ageing purpose [8-10]. *Olea europaea* contains Vit.E, Vit.K, thiamine, flavons, flavonols, which act as a good antioxidant, antiinflamatory, anti-ageing, photoprotective effect on skin [11-15].

Triticum astevum contains Vit.C, Vit.E, Vit.B1, B2, B3, B6, B12 etc. which act as antiacneic, used in many skin problems, antioxidant, antiageing [16-19].

The purpose of this study was to develop herbal sunscreen cream by mixing Daucuscarota oil, *Simmondsia chinensis* oil, *Triticum astevum* oil, *Olea europaea* oil that could produce sunscreen effect on skin.

Sun protection Factor

The efficacy of a sunscreen is usually expressed by the sun protection factor.SPF is stand for sun protection factor. Sun protection factor is defined as UV energy which is required to produce a Minimal Erythema Dose (MED) on protected skin, divided by the UV energy required to produce a MED on unprotected skin

$$SPF = \frac{\text{Minimal erythema dose on sunscreen protecte skin}}{\text{Minimal erythema dose in non sunscreen protected skin}}$$

It is necessary to standardize the methods which are used to determine the SPF of the sunscreen products.SPF of topical sunscreens against solar ultraviolet radiation exposure can be determined in vivo or in vitro[5,6].

***In Vitro* Determination of Sun Protection Factor**

There are two types of In-vitro method

Trans Pore Tape method-

It is a surgical tape manufactured by 3M company SPF is determined by using the PerkinElmer LAMBDA 1050 which is equipped with a 150 mm integrating sphere will be use to collect the scatter transmission data for sunscreen which is placed on a tape substrate. Testing of sunscreen on a tape model of human skin which is use to calculate the SPF value is more convenient and economical than testing on human skin. There were different brands of surgical tape were measured (in trans-mission) to determine which was the best brand representation of human skin. By using surgical tape sunscreen testing allows to be performed on a substitute for human skin, which is much safer than testing the product on actual skin.

Spectrophotometric evaluation

This method which involve the measurement of absorption or the transmission of UV radiation through sunscreen formulation. in which the absorption characteristics of the sunscreens agents are determined on spectrophotometric analysis of dilute solutions of sunscreen [20,21,22,23. Mansur et al. (1986), developed a very simple mathematical expression which substitutes the In Vitro method which is proposed by Sayre et al., (1979), Sun protection factor calculated by using the following equation and utilizing UV spectrophotometry

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where: EE (l) – erythemal effect spectrum;
I (l) – solar intensity spectrum;
Abs (l) - absorbance of sunscreen product
CF – correction factor (= 10).
The values of EE x I are constants.
This were determined by Sayre et al. (1979), and are showed below in Table 1.1

***In Vivo* Determination of Sun protection Factor**

Colipa Method

In this method UV radiation source with continuous emission spectrum 290-400 nm used as a source of radiation. The test product applied in concentration of 2mg/cm2 on the upper back of the volunteer 15 min prior exposure to radiation.

The UV sources should irradiate an area of 5.6cm in six point (diameter 1 cm), in series of doses increasing geometrically (factor 1.25).For the measurement and automatic delivery of radiation doses radiometer should be use. The visual evaluation of skin reaction should be done 16-24 h after UV exposure by a single trained evaluator .The minimal Erythema Doses on unprotected skin and protected skin by the test product for each test subject. The SPF of the sunscreen product should be calculated as erythematic mean of all subjects in the test.In accordance with ethical principles set in the declaration of Helsinki and International Ethical Guidelines for Biomedical Research Involving Human volunteer Subjects

MATERIAL AND METHOD

Wheat germ oil, carrot seed oil, olive oil and jojoba oil was purchased from the local market **Instruments**- Instruments used are TLC chamber, Brookfield viscometer, UV-spectrophotometer.

Chromatographic Analysis [26]

TLC profile of Jojoba oil, olive oil, Carrot seed oil and Wheat germ oil

In this TLC study, spots were applied on TLC plates. The spots of respective oils was applied on TLC plate and compared with the standard *RF* value result of herbal oils has been shown in Table no.4

Stationary Phase: Silica Gel G

Sample Preparation: Dissolve 1 ml of sample oil in 10 ml hexane.

Mobile Phase: Hexane-ethyl acetate (9 : 1, v/v)

Spraying Reagent: Iodine Vapors. After heating at 110° C for 5-10 minutes, the plate is evaluated in visible.

Quantitative Analysis

1. Specific Gravity:

Specific Gravity is the ratio of the density of a substance compared to the density (mass of the same unit volume) of a reference substance.

Water is used as Standard for specific gravity of liquid and solids.

Procedure:

Weight of empty specific gravity bottle is taken. The weight of specific gravity bottle filled with distilled water is taken. Weight of distilled water is determined by subtracting weight of empty bottle from weight of bottle filled with distilled at room temperature. Same procedure is followed for all three oils and specific gravity was determined.

Results are shown in table no.5

2. Saponification value:

The saponification value is the number of milligrams of potassium hydroxide necessary to neutralize the free acids and to saponify the esters present in 1 g of the substance.

Method

Unless otherwise specified in the individual monograph, introduce about 2 g of the substance under examination, accurately weighed, into a 200 ml flask of borosilicate glass fitted with a reflux condenser. Add 25.0 ml of 0.5 M ethanolic potassium hydroxide and a little pumice powder and boil under reflux on a water-bath for 30 minutes. Add 1 ml of phenolphthalein solution and titrate immediately with 0.5 M hydrochloric acid (a ml). Perform a blank determination omitting the substance under examination (b ml). The saponification value was been calculated by the expression. Results are shown in table no.6

Saponification value = 28.05 (*b* - *a*)/*w*

Where, w = weight, in g, of the substance.

3. Acid Value

The acid value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acids present in 1 g of the substance.

Method

Unless otherwise specified in the individual monograph, dissolve about 10 g of the substance under examination, accurately weighed, in 50 ml of a mixture of equal volumes of ethanol (95 per cent) and ether, previously neutralized with 0.1 M potassium hydroxide to phenolphthalein solution. If the sample does not dissolve in the cold solvent, connect the flask with a reflux condenser and warm slowly, with frequent shaking, until the sample dissolves. Add 1 ml of phenolphthalein solution and titrate with 0.1 M potassium hydroxide until the solution remains faintly pink after shaking for 30 seconds. The acid value has been calculated by the expression.

Acid value = 5.61 *n*/*w*

Where, n = the number of ml of 0.1 M potassium hydroxide required; w = the weight, in g, of the substance.

4. Ester Value

The ester value is the number of milligrams of potassium hydroxide required to saponify the esters present in 1 g of the substance.

Determine the acid value, and the saponification value, of the substance under examination. The ester value has been calculated by the expression.

Ester value = Saponification value - Acid value.

Determination of Sun Protection Factor of Herbal Oils [27]-

The absorption spectra of samples in solution were obtained in the range of 290 to 450 nm by using 1 cm quartz cell and n hexane as blank. The absorption data were obtained in the range of 290 to 320 every 5 nm and determination were made at each point, followed by the application of Mansur equation

Results of SPF of wheat germ oil, carrot seed oil, jojoba oil and olive oil have been shown in table no.7,8,9 and 10 respectively

EVALUATION OF OILS

Morphological Character

Qualitative Chemical Tests

Chromatographic Analysis

The presence of specific phytoconstituents with their approximate RF values were determined by conducting TLC of each oil as shown in figure 1.

Table 1. Normalized product function which are used in the calculation of SPF (Sayre et al., 1979)

Wavelength (λ nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Table 2. Morphological Character

Oil	Colour	Odour
Wheatgerm oil	Amber brown	Heavy
Carrotseed oil	Yellowish brown	Spicy and musky
Jojoba oil	Golden yellow	nutty like
Olive oil	Greenish yellow	Pleasant, agreeable

Table 3. Qualitative Chemical Tests

Parameters	Jojoba	Wheat germ	Olive	Carrot seed
Sudan Red Test	Red color	Red color	Red color	Red color
Solubility Test (90% alcohol)	Insoluble	Insoluble	Insoluble	Insoluble
Filter Paper Test	Permanent spot	Permanent spot	Permanent spot	Permanent spot

Table 4. Result of Thin Layer Chromatography (Rf Values of separated constituents)

Sr.No.	Olive oil	Carrot seed oil	Jojoba oil	Wheat germ oil
1	**0.15**	**0.22**	**0.29**	**0.22**
2	**0.24**	**0.32**	**0.35**	**0.33**

Table 5. Specific Gravity of Herbal Oils

Herbal Oil	Wheat germ oil	Carrot seed oil	Jojoba oil	Olive oil
Specific Gravity	**0.9257**	**0.917**	**0.861**	**0.910**

Table 6. Determination of Saponification value, Acid value and Ester value of oils

Parameter	Wheat germ Oil	Carrot seed Oil	Jojoba oil	Olive oil
Saponification value	191.22	173.91	88	190
Acid value	13.88	55.6	87	192.7
Ester value	177.34	118.5	85	190.86

Table 7. Determination of Sun Protection Factor of Wheat germ Oil

Wavelength (nm)	EE (λ) × I (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)
290	0.0150	3.522	0.05276
295	0.0817	3.553	0.30738
300	0.2874	0.942	0.27159
305	0.3278	1.056	0.34616
310	0.1864	3.116	0.58082
315	0.0839	3.219	0.27477
320	0.0180	3.009	0.06170
	Total = 1		Total= 2.24
			SPF= 22.40

Table 8. Determination of Sun Protection Factor of Carrot seed Oil

Wavelength (nm)	EE (λ) × I (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)
290	0.0150	3.522	0.05283
295	0.0817	3.553	0.29028
300	0.2874	0.942	0.27073
305	0.3278	1.056	0.34567
310	0.1864	3.116	0.59536
315	0.0839	3.219	0.27007
320	0.0180	3.009	0.05416
	Total = 1		Total= 1.88
			SPF=18.80

Table 9. Determination of Sun Protection Factor of Jojoba Oil

Wavelength (nm)	EE (λ) × I (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)
290	0.0150	1.2844	0.01926
295	0.0817	0.7062	0.05769
300	0.2874	0.4196	0.12059
305	0.3278	0.5290	0.19340
310	0.1864	0.3364	0.11862
315	0.0839	0.7526	0.05475
320	0.0180	0.3500	0.01770
	Total = 1		Total= 0.6022
			SPF= 6.022

Table 10. Determination of Sun Protection Factor of Olive Oil

Wavelength (nm)	EE (λ) × I (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)
290	0.0150	1.9640	0.02954
295	0.0817	1.5070	0.12312
300	0.2874	0.8161	0.23460
305	0.3278	0.8270	0.27129
310	0.1864	0.9610	0.18116
315	0.0839	0.8014	0.07429
320	0.0180	0.8045	0.01449
	Total = 1		**Total = 0.9285**
			SPF=9.285

Figure 1. TLC of Olive oil, Carrot seed oil, Jojoba oil and Wheat germ oil

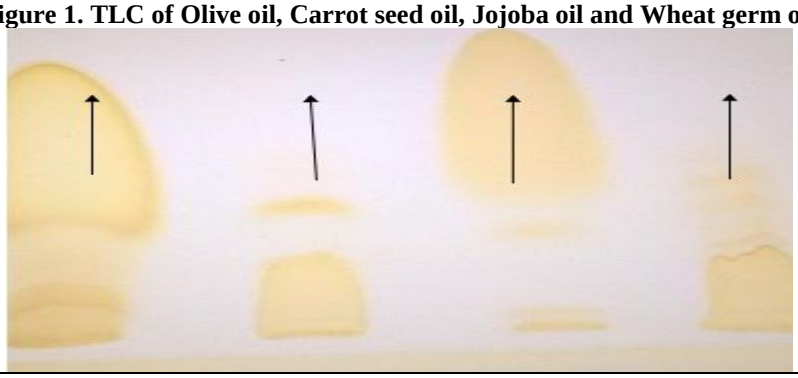

Fig.2.TLC of Olive oil	Fig.3. TLC Carrot seed oil	Fig.4. TLC Jojoba oil	Fig.5. TLC Wheat germ oil
			

CONCLUSION

Sun Protection Factor is the quantitative measurement of the effectiveness of the sunscreen formulation. From the results obtained it is evident that all this four oil may be considered as good candidate for sunscreen or cosmoceutical purpose. All this four oil has good sunscreen activity and can be considered as active sunscreen agent and anti ageing agent or can be incorporated into other sunscreen formulations as an additive to enhance the activity. From the above discussion it is concluded that on combining the all oil in different ratio**,** it can be possible to increase the efficacy of sunscreen activity of herbal oils as compared to single oil. In this regard when we would be mix the carrot seed oil, wheat germ oil, olive oil and jojoba oil in different ratio to get multipurpose effect such as whitening, anti wrinkle, anti aging and sunscreen effect on skin so this study will be helpful for the researcher to develop potential sunscreen formulation and multipurpose skin formulation. From the above it concluded that this herbal oil produce excellent whitening, anti wrinkle and potential sunscreen effect on skin.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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